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Investigating Antivenom Function and Cross-Reactivity – a Study of Antibodies and Their Targets

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Snake Antivenom: an Essential Medicine – and a Black Box

Venomous snakebites are regarded as one of the World's most neglected tropical diseases/conditions with up to 2.5 million victims every year[1]. The best-practice treatment is antivenom derived from the blood of large mammals (typically horses or sheep) immunized with venom of one or more snake species. The active toxin-neutralizing components in antivenom are complex mixtures of antibodies (or fragments hereof)[2]. The individual antibodies are adapted by the immune system of the production animal to bind specific to parts of each toxin used in the immunization procedure. In many cases antivenom is also able to neutralize some – or even all – toxic effects of snakebites from related snake species[3].

Proteomics-based studies aiming at quantifying the extent of such cross-protection of antivenoms against venoms from related snake species are referred to as antivenomics. The current state-of-the-art antivenomics protocol involves affinity chromatography of venoms with immobilized antibodies[4]. Although proven effective in clinical applications antivenomics fail to explain how this cross-reactivity is working at the molecular level and must be performed for one snake venom-antivenom pair at a time.

Knowledge of interactions between the immunoreactive parts (referred to as epitopes) of a toxin or macromolecule in general and the corresponding antibodies is a prerequisite to understand and predict neutralization potential of a given antivenom against any fully characterized snake venom. Although antivenom to snakebites is a more than 120 years old invention[1], only little is known about the neutralizing antibodies or their epitopes[5].

Ideas and Perspectives

- Identify linear peptides from snake toxins that can bind antibodies in antivenom using custom designed high-density peptide microarray technology[6]. See figure 1
- The microarrays in this study have been designed to contain five technical replicates of 93'261 15-mer peptides derived 966 toxins from pit viper snake species (sub-family Viperidae).
- Localize epitopes in peptide hits
- Characterizing important antibody-toxin interactions based on allowed variation of epitope
- Predict cross-reactivity of antivenoms on a protein family level and thereby expand the clinical applications of existing antivenoms to other snake species or suggest changes in immunization mixture to improve the medicine
- Learning from nature's preferences for specific epitopes, it will be possible to estimate the number of antibodies needed to neutralize the critical toxins for any given snake species
- In the long run this may result in recombinant immunization mixtures and even lead to the first fully recombinant antivenom

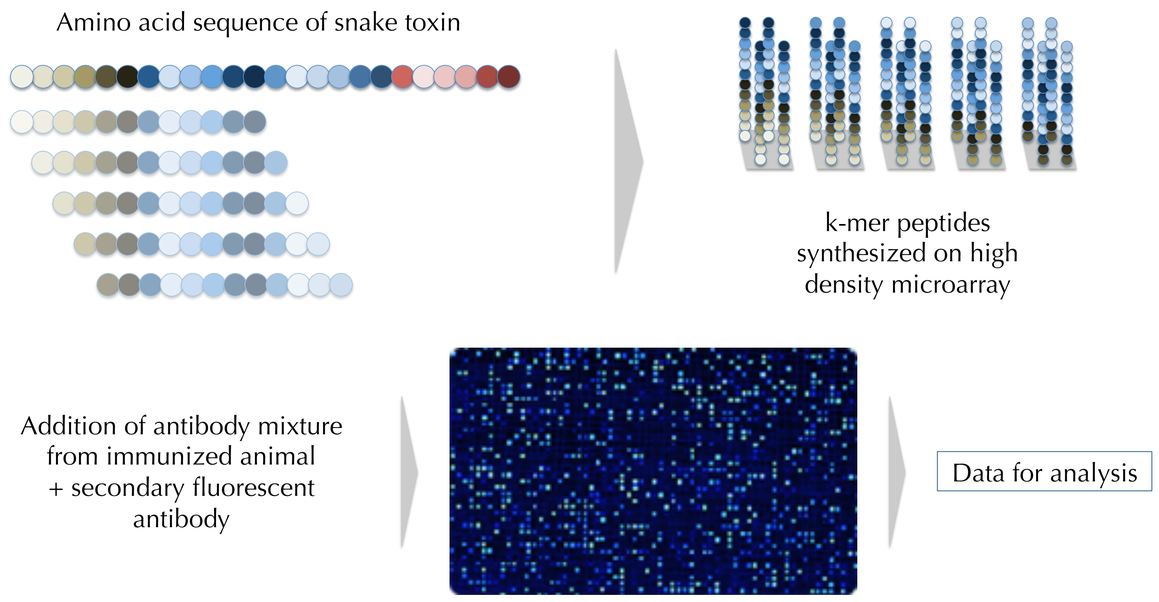

Figure 1 – Schematic overview of principle in peptide microarray experiments

Result example – Venom Metalloproteinase P-I subfamily

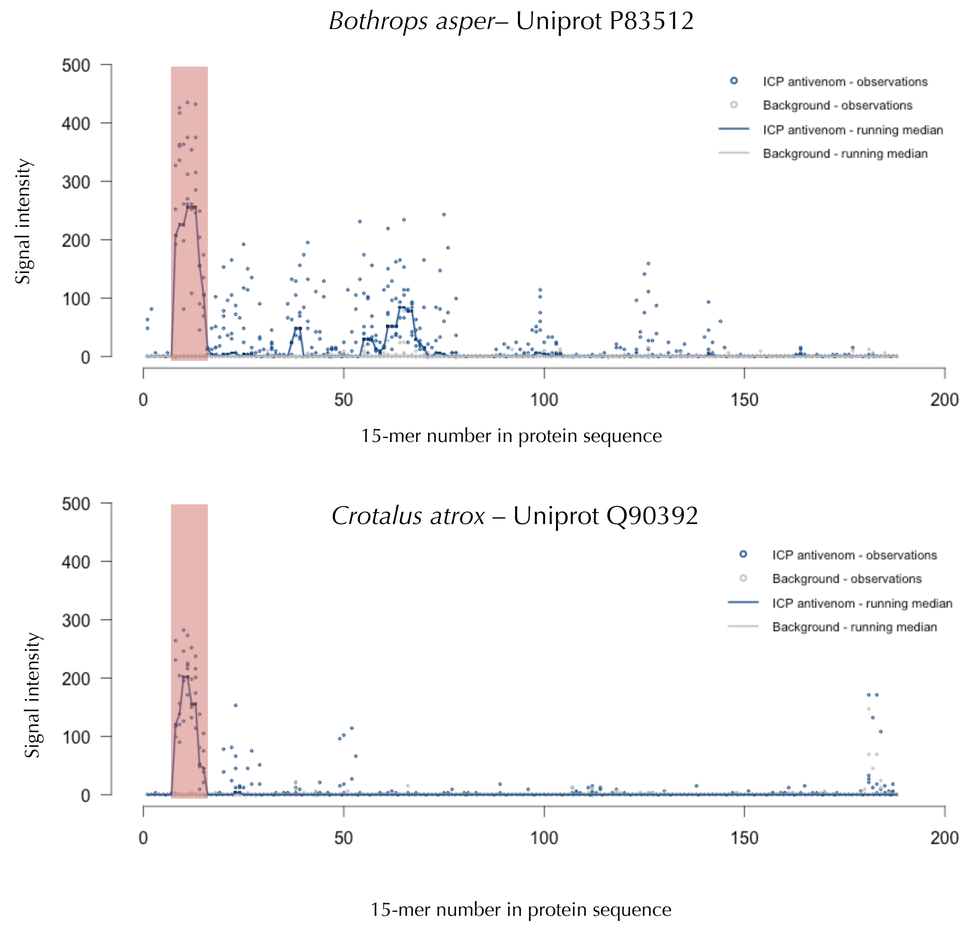

Figure 2 – Signal plots for two snake venom metalloproteinases (SVMP) belonging to the P-I sub-family. Peaks in the signal plots result from binding events between consecutive peptides from the protein sequence of toxins and antibodies in ICP anti-Bothrops antivenom. *Bothrops asper* venom was used in the immunization procedure and the SVMP in the upper plot is known to represent approx. 30 % of the total protein composition in venom from adult specimens7. The SVMP from *Crotalus atrox* displayed the lower plot is not present in the immunization mixture but shares 60 % of the amino acids with the *B. asper* SVMP. The lower plot strongly indicates a cross-neutralization potential of the ICP antivenom.

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#	Sequence	Signal
7	RYIELAVVADHGIFT	0
8	YIELAVVADHGIFTK	207
9	IELAVVADHGIFTKY	225
10	ELAVVADHGIFTKYN	225
11	LAVVADHGIFTKYNS	255
12	AVVADHGIFTKYNSN	255
13	VVADHGIFTKYNSNL	255
14	VADHGIFTKYNSNLN	156
15	ADHGIFTKYNSNLNT	105
16	DHGIFTKYNSNLNTI	12
	ADHGIFTK	

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#	Sequence	Signal
7	RYIELVVVADHRVFM	0
8	YIELVVVADHRVFMK	120
9	IELVVVADHRVFMKY	138
10	ELVVVADHRVFMKYN	201
11	LVVVADHRVFMKYNS	201
12	VVVADHRVFMKYNSD	156
13	VVADHRVFMKYNSDL	156
14	VADHRVFMKYNSDLN	51
15	ADHRVFMKYNSDLNT	45
16	DHRVFMKYNSDLNTI	0
	ADH**RVFM**K	

Figure 3 – Alignment of peptide 7 to 16 of the SVMPs from the signal plots in figure 2 including the running median signal score calculated as median of signals from peptide and the nearest neighbors on each side. The binding core is highlighted with red squares.

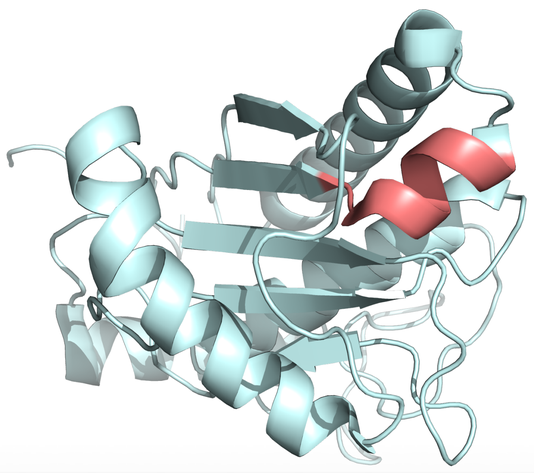

Figure 4 – Crystal structure of *B. asper* SVMP-PI (PDB entry 2W15). The α-helix highlighted in red correspond to the binding core of one or more antibody from the ICP antivenom

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